IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Eiji MORI *et al.*
Title:	ANTI-TRAIL-R ANTIBODY
Appl. No.:	10/721,763
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Examiner:	Claire M. Kaufman
Art Unit:	1646
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DECLARATION UNDER 37 CFR §1.132

Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Kazuhiro Motoki, declare that

1. I am a citizen of Japan and am employed as a Senior Scientist in the Discovery Research Laboratories of KIRIN PHARMA COMPANY, LTD., which is located at 3 Miyahara-cho, Takasaki-shi, Gunma, 370-1295, Japan. I graduated from Graduate School of Pharmaceutical Sciences, Chiba University with a master's degree in 1991, and obtained a PhD at Niigata University in 1996. At KIRIN PHARMA COMPANY, I studied the tumor immunology for more than 15 years. In detailed, I studied the antitumor efficacy and mechanisms of action of novel immunomodulator, α-galactosylceramide, from 1992 through 2000. For last 7 years, I studied the antitumor efficacy and mechanisms of action of anti TRAIL-R2 mAb.

2. I am a co-inventor of U.S. application serial No. 10/721,763, "*Anti-Trail-R Antibody*," filed on May 17, 2002. I would get a predefined amount of remuneration from Kirin Pharma Company when the present application is granted. Further, it is expected that I would be remunerated when Kirin Pharma Company gains an income in commercializing a product or technology that is related to the invention of the present application.

3. I have read the U.S. Patent & Trademark Office's Office Action dated September 27, 2007, and publications cited there: Griffith *et al.*, *J. Immunol.* 162:2597 (1999); U.S. Patent No. 6,342,369; and U.S. 2003/0190687. I understand that Examiner Kaufman believes that certain prior-art antibodies, M413, 16E2, and TRA-8, can induce apoptosis when they are in monomeric form.

4. We conducted experiments on 16E2, and TRA-8 and concluded that none of these antibodies are a monoclonal antibody or antibody fragment that binds to TRAIL-R2 to induce apoptosis in carcinoma cells in a monomeric form. Rather, the studied antibodies only induced apoptosis when they were aggregated into a polymeric complex. The following paragraphs relate the results of the experiments in question, which concerned16E2, and TRA-8.

5. **16E2**: Examiner Kaufman likewise believes, I understand, that the 16E2 antibody induces apoptosis in carcinoma cells (SK-MES-1) when it is not cross-linked. The 16E2 antibody is a single-chain Fv (scFv), as described in U.S. Patent No. 6,342,369 (column 11, lines 41 to 55). Generally, scFv forms a polymer without cross-linking, as demonstrated, for instance, by Kortt *et al.*, *Protein Engineering* 10: 423, 1997 (appended). In particular, Kortt showed by gel filtration that affinity purified scFv antbodies yielded not only monomeric antibodies but also dimers and trimers. It should be notable that affinity purified scFv antibodies contained the approximate half amount of polymers (see Kortt's Figures 1 and 5).

6. We conducted experiments that demonstrated that a preparation of 16E2 antibodies contained a extremely large amount of polymeric forms of the antibody. More specifically, I produced the 16E2 antibody according to the method disclosed in U.S. Patent No. 6,342,369, and I then examined whether that antibody formed a polymer. Exhibit A to this declaration shows that almost all of the 16E2 antibodies formed dimeric and tetrameric polymers. I detected very little amount of monomeric 16E2, if any. Accordingly, I conclude that the apoptosis-inducing effects of the 16E2 antibody are due to its polymeric form; hence, that 16E2 does not function as "a single substance without polymer."

7. <u>TRA-8</u>: I understand Examiner Kaufman also to say that the TRA-8 antibody, when it is not cross-linked, induces apoptosis in Jurkat leukemia cells. Pursuant to testing detailed in Exhibit B to this declaration, however, I determined that the monomeric TRA-8 antibody did not induce apoptosis without cross-linking. More specifically, I found that the TRA-8 antibody had no apoptotic activity when it existed in culture medium but that TRA-8 could induce apoptosis only when it was cross-linked (see "Experiment 1" of Exhibit B). In

addition, I demonstrated that purified TRA-8 antibody contained both antibody monomer and polymer (see "Experiment 2"). I also found that the purified TRA-8 antibody had apoptosis-inducing activity without any cross-linker (see "Experiment 3"). Furthermore, I isolated the TRA-8 antibody monomer ("Experiment 4") and found that the antibody monomer did not have any apoptosis-inducing activity ("Experiment 5"). I conclude, therefore, that the TRA-8 antibody monomer does not induce apoptosis but that only the polymeric form of TRA-8 induces apoptosis in the absence of any cross-linker.

We also evaluate Griffith's M413 antibody as follows.

8. **M413**: Examiner Kaufman believes, I understand, that Griffith's M413 antibody induces apoptosis in carcinoma cells when it is not cross-linked, *i.e.*, when it is not "polymeric." Even without cross-linking, however, antibodies themselves generally form an aggregate spontaneously. For instance, we had demonstrated in Example 27 of the application that a purified antibody preparation contained both monomeric and polymeric forms of the antibody. See also our Example 28, which showed that the monomeric "0304" and "0322" antibodies fractionated by gel filtration chromatography exhibited cell-death-inducing activity against Colo205 cells without any cross-linker, but that the monomeric "H-48-2" antibody had no cell-death inducing activity on Colo205 cells. Thus, the polymeric form of H-48-2 antibody only exhibited cell-death-inducing activity in the absence of any cross-linkers. For that reason it is possible that any apoptosis reported by Griffith was due to a naturally-aggregated polymeric fraction of the M413 antibody preparation, and not to a 100% preparation of a monomeric form of the antibody. We could not specifically test M413 antibody because it is not available to the public; that is, the M413 antibody is not commercially available and has not been placed in a depository institution. Accordingly, it was impossible for us to evaluate the structural and functional characteristics of M413.

9. In summary, I conclude that none of the prior-art antibodies can induce apoptosis in monomeric form. We found that the prior-art antibodies were able to induce apoptosis only when they formed polymeric antibody aggregates.

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I hereby declare that all the statements made herein of my known knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements are so made punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United

States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

March 28/2008
DATE

Kazuhiro Motoki
KAZUHIRO MOTOKI